Clinical study report

Synopsis of the clinical study

Sponsor:	University of Antwerp Universiteitsplein 1 2610 Wilrijk, Belgium	Represented by: Prof. Dr. Nina Hermans
Title of study:	Effect of Pycnogenol® on Attention-Deficit Hyperactivity Disorder (ADHD): A randomized, double-blind, placebo and active product controlled multicenter trial.	
Type of trial, trial design and methodology:	Phase III, multicenter randomized double-blind placebo-controlled parallel group design with three treatment arms (French Maritime Pine Bark Extract (Pycnogenol®, PBE), Methylphenidate (MPH) and placebo)	
Publications:	▪ Verlaet AAJ, Ceulemans B, Verhelst H, et al. (2017) Effect of Pycnogenol® on attention-deficit hyperactivity disorder (ADHD): study protocol for a randomised controlled trial. *Trials 2017 18:1* 18(1). BioMed Central: 1–9. DOI: 10.1186/S13063-017-1879-6. (Peer-reviewed publication of trial protocol) ▪ Weyns A-S, Verlaet AAJ, Breynaert A, et al. (2022) Clinical Investigation of French Maritime Pine Bark Extract on Attention-Deficit Hyperactivity Disorder as compared to Methylphenidate and Placebo : Part 1 : Efficacy in a Randomised Trial. *Journal of Functional Foods* 97 (September). Elsevier Ltd: 105246. DOI: 10.1016/j.jff.2022.105246. (Peer-reviewed publication of trial results) ▪ Weyns A-S, Verlaet AAJ, Herreweghe M van, et al. (2022) Clinical Investigation of French Maritime Pine Bark Extract on Attention-Deficit Hyperactivity Disorder as compared to Methylphenidate and Placebo : Part 2 : Oxidative Stress and Immunological Modulation. *Journal of Functional Foods* 97 (September). Elsevier Ltd: 105247. DOI: 10.1016/j.jff.2022.105247. (Peer-reviewed publication of trial results)	
Study period:	September 2017 – November 2020	
Number of subjects planned:	48 per treatment group were to be included (total n= 144)	
Number of subjects analysed:	Placebo (n = 30), PBE (n = 32) or MPH (n = 26)	
Principal inclusion criteria:	▪ 6-12 years old (both inclusive) ▪ Diagnosis of ADHD/ADD ▪ Responsible caregiver able to provide information about the patient's functional status ▪ Sufficient level of Dutch ▪ Written informed consent from the legally accepted representative	
Principal exclusion criteria:	▪ Diagnosis of Autism Spectrum Disorder (ASD) ▪ Situational hyperactivity, pervasive developmental disorder, schizophrenia, personality disorder, Intelligence Quotient (IQ) < 70, conduct disorder (CD), dyskinesias, tics or Tourette's syndrome, personal/family history of psychotic disorder, bipolar illness, depression or suicide attempt ▪ Any chronic medical disorder or acute inflammatory disease, glaucoma, heart disease or rhythm disorder, high blood pressure or peripheral vascular disease ▪ Contraindications for the use of MPH ▪ Pregnancy ▪ Use of any of these medications during the past 3 months: clonidine, guanethidine, blood thinners, antidepressants, medication with decongestant, blood pressure medicine, seizure medicine or diet pills ▪ Use of Monoamine oxidase (MAO) inhibitor in the past 2 weeks	

	▪ Use of vitamin/mineral/herbal/omega-3 supplements or any medication >1 week during the past 3 months
Objectives:	The objective of this trial was to evaluate effects of the polyphenol-rich French Maritime Pine Bark Extract (PBE; Pycnogenol, Horphag Research) on ADHD behaviour, co-morbid physical/psychiatric symptoms, immunological markers, and oxidative stress and neurochemical status, compared to placebo and methylphenidate (MPH). Moreover, effects of the described treatments on gut microbial composition have been evaluated in a participant subgroup.
Study end points:	<u>Primary end point</u>: ▪ Summed ADHD score of the ADHD Rating Scale (ADHD-RS) rated by teachers <u>Secondary end points:</u> ▪ Summed ADHD score of the ADHD-RS rated by parents ▪ Summed ADHD score of the Social-Emotional Questionnaire (SEQ) rated by parents and teachers ▪ Percentage of responders <u>Other variables:</u> ▪ Social behaviour problems and anxiety subscales of the SEQ rated by parents and teachers ▪ Physical and sleep complaints measured by the Physical Complaints Questionnaire (PCQ) rated by parents ▪ Erythrocyte glutathione (GSH) levels ▪ Lipid soluble antioxidant status (α- and γ-tocopherol, β-carotene, retinol and co-enzyme Q10) ▪ Antioxidant enzyme activity: catalase (CAT), superoxide dismutase (SOD) en glutathione peroxidase (GPX) ▪ Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels ▪ Plasma malondialdehyde (MDA) level ▪ Plasma cytokine level (IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF- α, IFN-γ) ▪ Plasma antibody level (IgA, IgG1-4, IgE) ▪ Intestinal microbial composition ▪ Gene expression quantification by real-time PCR (RT-qPCR) of genes GPX, CAT, SOD, XO) and Apolipoprotein J (ApoJ) ▪ Serum neuropeptide Y (NPY) level ▪ Serum zinc level ▪ Acceptability: dropouts, adherence and adverse events
Name investigational medicinal product (IMP), posology and administration:	PBE, oral intake 20 mg/day if body weight < 30 kg 40 mg/day if body weight ≥ 30 kg First 2 weeks: all participants 20 mg/day
IMP or therapy used as comparator, posology and administration:	Placebo, oral intake Medikinet® Retard (MPH), oral intake First week: 10 mg/day MPH Second week: 20 mg/day MPH Afterwards: 20 mg/day MPH if body weight < 30 kg, 30 mg/day MPH if ≥ 30 kg
Duration of treatment:	10 weeks
Statistics	SPSS 27.0 (IBM) and R version 4.1.1 (R core team) were used for statistical analyses

Results

Primary end point

Based upon teacher-rated ADHD-RS, the primary outcome, MPH treatment caused significant improvement in total and inattention score as compared to placebo after 5. After 10 weeks, both PBE and MPH significantly improved the total and hyperactivity/impulsivity score, while MPH also improved inattention.

Secondary endpoints

- SEQ ratings largely confirm ADHD-RS results. For teacher and parent ratings, no significant difference in effects between the three treatments was found regarding SEQ autism, social problem behaviour and anxiety (sub)scores.
- PBE and MPH did not affect co-occurring psychiatric and physical complaints No serious adverse reactions were reported. However, nonserious side effects were reported five times more frequently for MPH than for PBE ($p<0.01$). Side effects reported for PBE were headache, dizziness, nausea and diarrhea; for MPH GI symptoms, reduced appetite, insomnia, headache, a feeling of tachycardia, sneezing and being emotional have been noticed.
- No significant differences between treatments were found for erythrocyte GSH level, plasma retinol, α- and γ-tocopherol, β-carotene and co-Q10, GPX and SOD enzyme activity, and GPX, CAT, SOD and XO gene expression (markers of antioxidant status), nor for plasma MDA and urinary 8-OHdG levels (markers of oxidative damage), serum zinc level, and plasma IgE, IgG1, IgG3, IgG4, IL-1β, IL-4, IL-5, IL- 6, IL-8, IL-10, IL-12, IFN-γ and TNF-α levels and Apolipoprotein J gene expression (markers of immune status).
- After 10 weeks, MPH treatment significantly increased IgA and IgG2 levels as compared to PBE.
- A significant decrease in CAT activity after treatment with PBE was demonstrated as compared to placebo.
- No significant change in MDA levels between groups was observed.
- 8-OHdG concentration in the placebo group seemed to have increased (although not significantly) at the end of the study
- Serum NPY levels, together with body weight, were significantly decreased after 10 weeks MPH, in contrast to unaltered NPY and an expected physiological weight gain with PBE.

Limitations

Although 144 patients were to be included based on power calculation, the trial was ended with 88 participants in November 2020 due to poor inclusion and missing teachers questionnaires (as a result of homeschooling) during COVID-19 pandemic in combination with expiry of study capsules. Despite reduced power, enough patients were included to draw conclusions by using linear mixed models (LMM) analysis and post-hoc testing. Microbiome analysis was also impeded during the Covid-19 pandemic in which SARS-CoV2 analysis was prioritized in PCR facilities.

Conclusions

In paediatric ADHD and especially in the school environment, PBE was proven to be a good alternative for MPH for those willing to wait a few weeks for effects, a fortiori when taking into account its almost complete lack of side effects as opposed to MPH. These results should ideally be confirmed by future trials involving a greater number of patients, providing more information on specific subgroups, dosing and mechanisms of action.

As described on page 1 of this report, results of this trial have been published in 2 research papers (DOI: 10.1016/j.jff.2022.105246 and DOI: 10.1016/j.jff.2022.105247)